Adventist Agricultural Association
4th Annual Convention
2018
Session 1

Nitrogen Cycle

PRESENTED BY:

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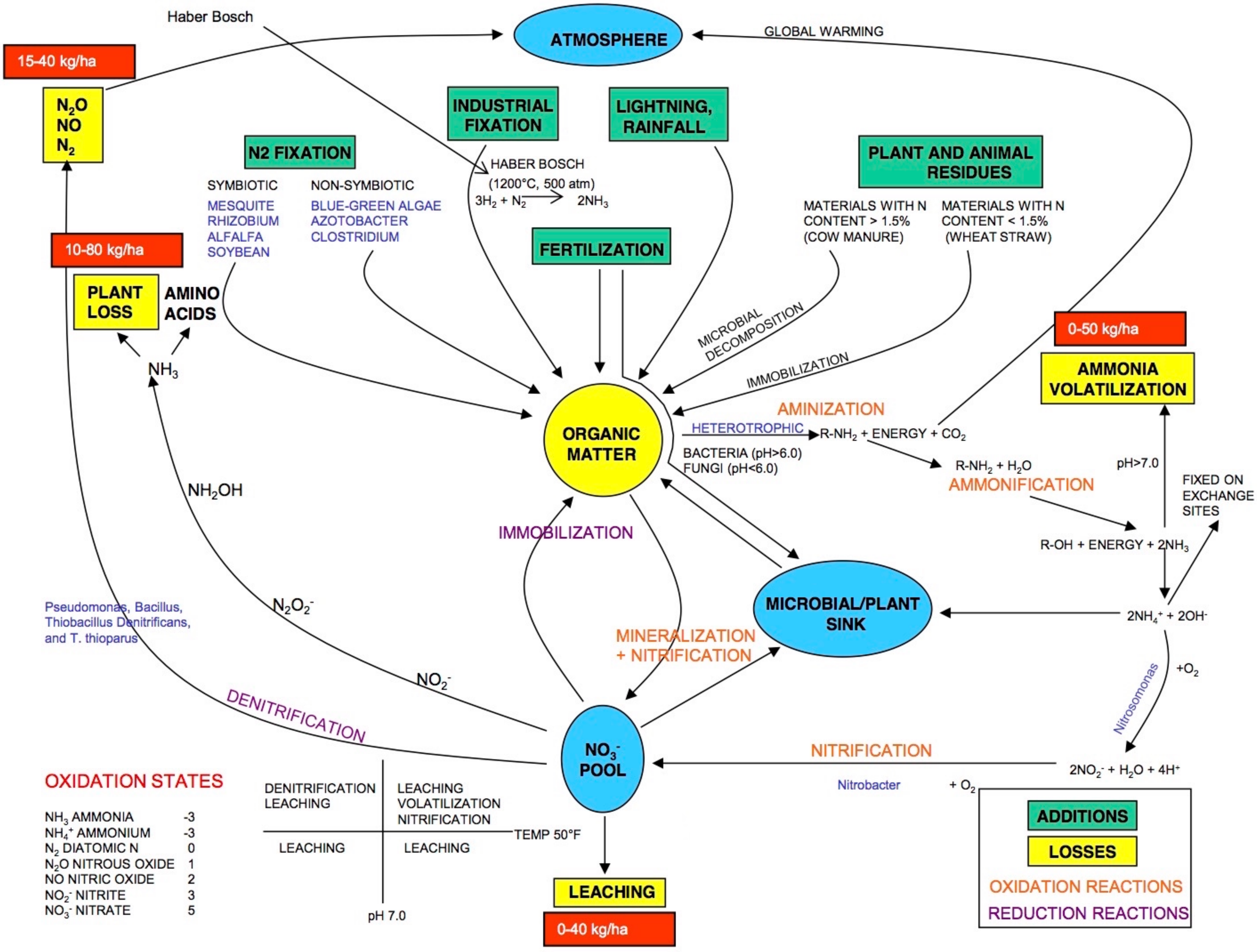


Haber Bosch
ATMOSPHERE
GLOBAL WARMING
15-40 kg/ha
N2O
NO
N2
INDUSTRIAL FIXATION
LIGHTNING, RAINFALL
N2 FIXATION
PLANT AND ANIMAL RESIDUES
SYMBIOTIC
NON-SYMBIOTIC
HABER BOSCH
(1200°C, 500 atm)
3H2 + N2 → 2NH3
MESQUITE
RHIZOBIUM
ALFALFA
SOYBEAN
BLUE-GREEN ALGAE
AZOTOBACTER
CLOSTRIDIUM
MATERIALS WITH N CONTENT > 1.5% (COW MANURE)
MATERIALS WITH N CONTENT < 1.5% (WHEAT STRAW)
10-80 kg/ha
FERTILIZATION
PLANT LOSS
AMINO ACIDS
MICROBIAL DECOMPOSITION
IMMOBILIZATION
0-50 kg/ha
AMMONIA VOLATILIZATION
NH3
AMINIZATION
HETEROTROPHIC
ORGANIC MATTER
R-NH2 + ENERGY + CO2
BACTERIA (pH>6.0)
FUNGI (pH<6.0)
R-NH2 + H2O
AMMONIFICATION
pH>7.0
FIXED ON EXCHANGE SITES
NH2OH
IMMOBILIZATION
R-OH + ENERGY + 2NH3
Pseudomonas, Bacillus, Thiobacillus Denitrificans, and T. thioparus
N2O2-
MICROBIAL/PLANT SINK
2NH4+ + 2OH-
MINERALIZATION + NITRIFICATION
Nitrosomonas
+O2
NO2-
DENITRIFICATION
NITRIFICATION
NO3- POOL
2NO2- + H2O + 4H+
Nitrobacter
+ O2
OXIDATION STATES
DENITRIFICATION LEACHING
LEACHING VOLATILIZATION NITRIFICATION
TEMP 50°F
LEACHING
LEACHING
pH 7.0
NH3 AMMONIA -3
NH4+ AMMONIUM -3
N2 DIATOMIC N 0
N2O NITROUS OXIDE 1
NO NITRIC OXIDE 2
NO2- NITRITE 3
NO3- NITRATE 5
LEACHING
0-40 kg/ha
ADDITIONS
LOSSES
OXIDATION REACTIONS
REDUCTION REACTIONS

Forms of soil N

- Elemental (N_2)
  - Gas in soil atmosphere
  - Dissolved in soil water
  - Symbiotic and non-symbiotic fixation
    - Generates NH_4^+
- Inorganic forms
  - Gases –present in small amounts in soils
    - N_2O, NO, NO_2, **NH_3**
  - Ionic forms
    - NH_4^+ -exchangeable and non-exchangeable forms
    - NO_3^-, NO_2^-
  - Organic forms -98% of total soil N

N Transformations and cycling in soils

N Gains	N losses	N Cycling (within soil, N not lost)
Fixation (biological or mechanically – fertilizer)	Plant Uptake	Immobilization
Animal manure	Denitrification	Mineralization
Crop residue	Volitization	Nitrification
	Leaching	
	Ammonium fixation	

Vocabulary

- Nitrification: Conversion of NO_2 to NO_3
- Denitrification: Conversion of NO_3 to N gas
- Immobilization: Conversion of PAN to Plant unavailable N
- Nitrogen Fixation: Non Organic/Non Mineral forms of N to Organic forms of N
- Mineralization: The conversion of an element from an organic from to an inorganic state as a result of microbial decomposition.

N Mineralization

- Conversion of organic N to NH_4^+ through two reactions:
  - **aminization:** converts proteins in residues to *amino acids*, *amines*, and *urea*
  - **ammonification:** further converts organic N compounds to inorganic NH_4^+
    - The NH_4^+ produced is subject to several fates:

      - Nitrification

      - N uptake

      - immobilization

      - NH_4^+ fixation

      - Volatilization

N Immobilization

- Conversion of inorganic N (NH_4^+ and NO_3^-) to organic N
  - If decomposing residues contain low N, microorganisms will immobilize NH_4^+ and NO_3^- in the soil solution
  - C:N ratio

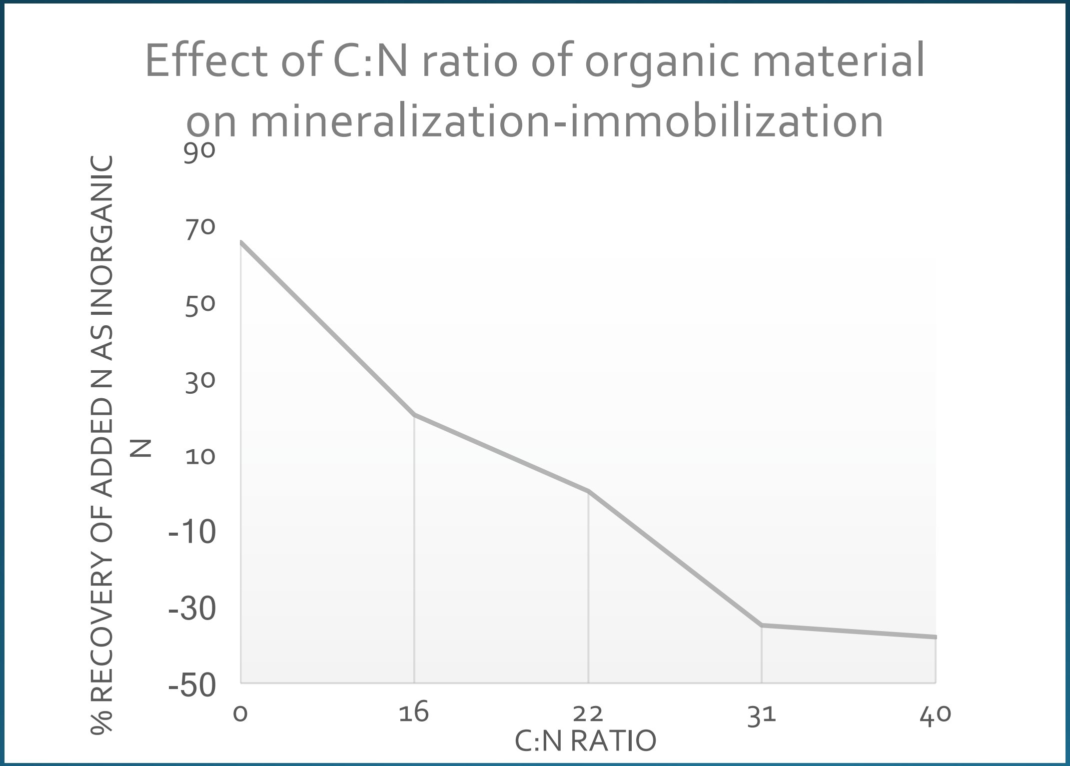


TABLE 16–2 Validly Described Genera and Species of Root-Nodule Bacteria of Legumes. Changes to this taxonomy suggested by Young et al. (2001) are in bold type. Genera in the square brackets refer to better-known host legumes nodulated by each species of root-nodule bacteria. Common names are included for well-known legume genera. In several examples in this list, different species of root-nodule bacteria nodulate the same legume.*

*Azorhizobium***
- *A. caulinodans [Sesbania]*

Bradyrhizobium
- *B. elkanii [Glycine*, soybean]
- *B. japonicum [Glycine]*
- *B. liaoningense [Glycine]*
- *B. yuanmingense [Lespedeza]*

Mesorhizobium
- *M. amorphae [Amorpha]*
- *M. chacoense* [*Prosopis*, mesquite]
- *M. ciceri* [*Cicer*, chickpea]
- *M. huakuii* [*Astragalus*, milkvetch]
- *M. loti [Lotus]*
- *M. mediterraneum [Cicer]*
- *M. plurifarium* [*Acacia, Leucaena*, Ipil-ipil]
- *M. tianshanense [Glycyrrhiza, Sophora]*

Rhizobium
- *R. etli* [*Phaseolus vulgaris*, bean]
- *R. galegae [Galega, Leucaena]*
- *R. gallicum [Phaseolus, Dalea, Onobrychis, Leucaena]*
- *R. giardinii [Phaseolus]*
- *R. hainanense [Stylosanthes, Centrosema]*
- *R. huautlense [Sesbania]*
- *R. indigoferae [Indigofera]*
- *R. leguminosarum*
  - *bv trifolii* [*Trifolium*, clover]
  - *bv viciae* [*Pisum*, peas, *Vicia*, field beans, *Lathyrus; and Lens*, lentil]
  - *bv phaseoli* [*Phaseolus*]
- *R. loessense [Astragalus]*
- *R. mongolense [Medicago, Phaseolus]*
- *R. sullae [Hedysarum]*
- *R. tropici* [*Phaseolus; Leucaena, Dalea, Macroptilium*]

Allorhizobium
- *A. undicola*, ***R. undicola*** *[Neptunia]*
- ***R. radiobacter*** [non-nodulating saprophyte], ***R. rhizogenes*** [causes hairy root disease],
- ***R. rubi, R. vitis***

Sinorhizobium
- *S. abri [Abrus]*
- *S. americanus [Acacia]*
- *S. arboris*
- *S. fredii [Glycine]*
- *S. indiaense [Sesbania]*
- *S. kostiense*
- *S. kummerowiae [Kummerowia]*
- *S. medicae [Medicago]*
- *S. meliloti* [*Melilotus*, sweetclover; *Medicago*, alfalfa; *and Trigonella*, fenugreek]
- *S. morelense [Leucaena]*
- *S. saheli, S. sahalense [Sesbania]*
- *S. terangae [Sesbania, Acacia]*
- *S. xinjiangense [Glycine]*

*Other genus and species names exist in the literature. Some predate the present names; others have not been accepted as valid.

**Strains which have not yet been recognized as belonging to any named species are usually identified by the host from which they were isolated—for example, *Rhizobium* spp. (*Acacia*) or *Bradyrhizobium* spp. (*Lupinus*).

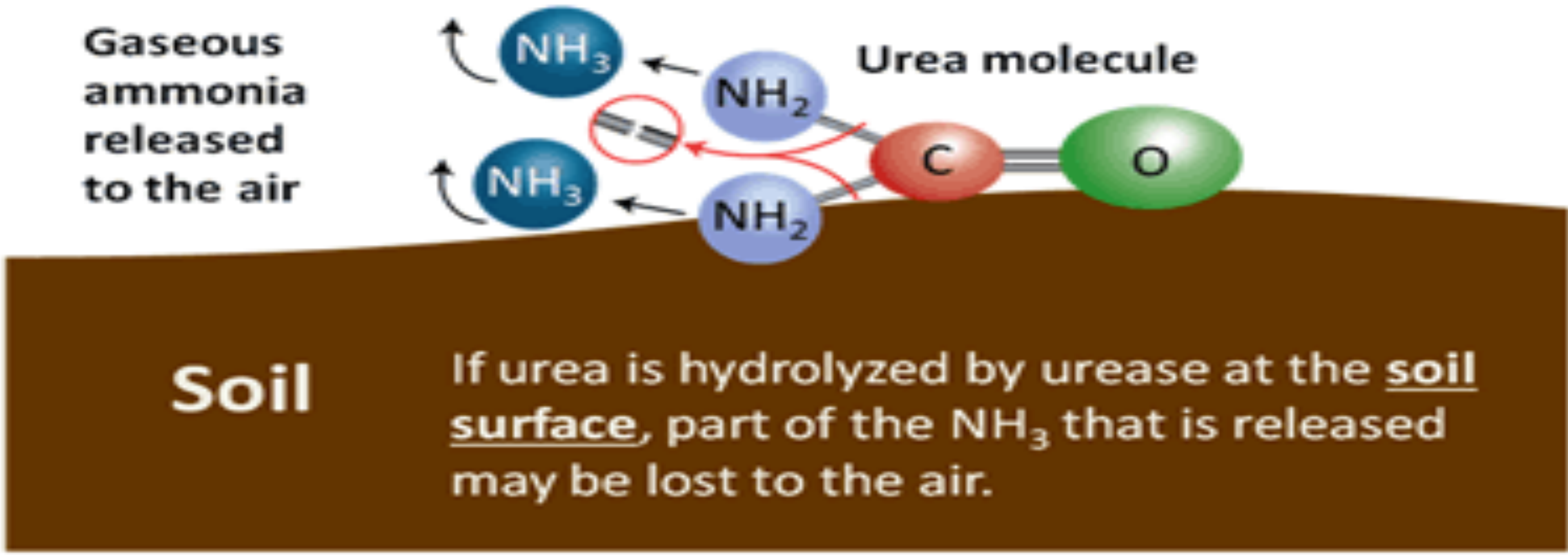
Events Leading to Nodulation and Dinitrogen Fixation in Legumes

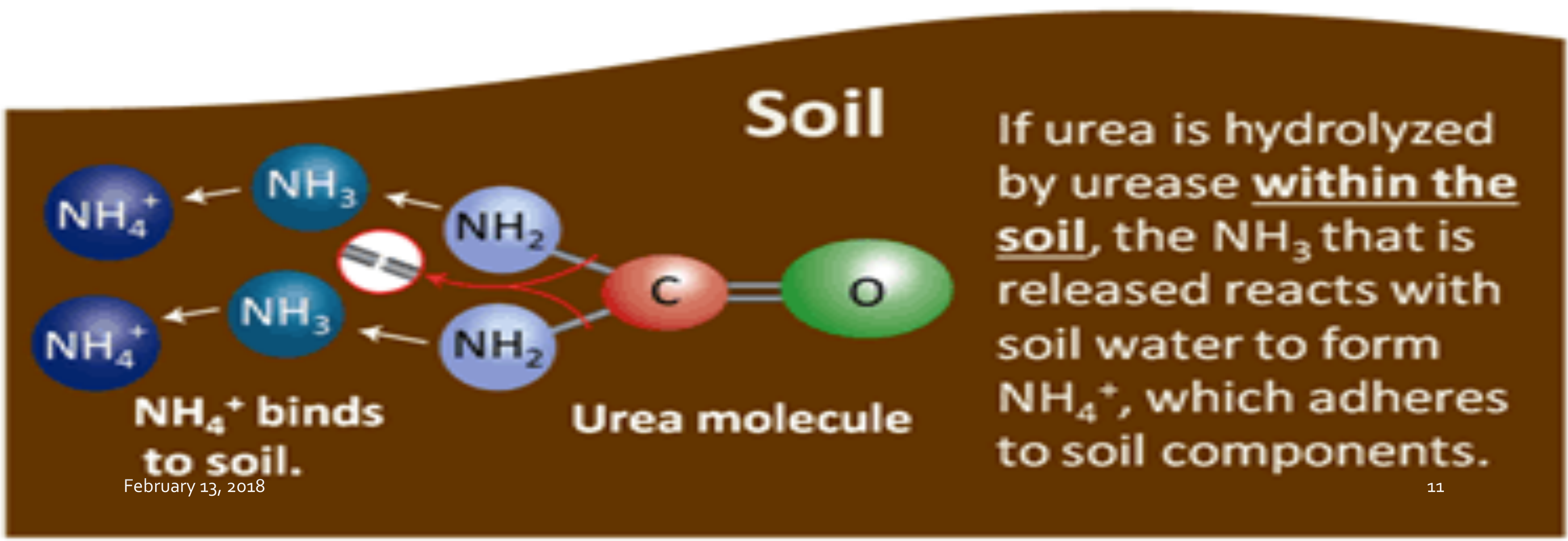
- Attachment of rhizobia to the root begins within 1 minute of inoculation.
- Number of attached rhizobia increases with time up to several hours.
- Root hair curling begins within 5 hours.
- Infection threads visible in the root hair within 3 days of inoculation.
- Nodule become visible within 5 to 12 days.
- N_2 fixation is often evident in 15 day-old plants.

N use and fertility

- Global use of manufactured N fertilizers has increased by a factor of 8 over past six decades

- Efficiency of fertilizer N is frequently low
  - Often <50% of applied N is taken up by crop
  - For recommended rates 50-70% efficiency assumed

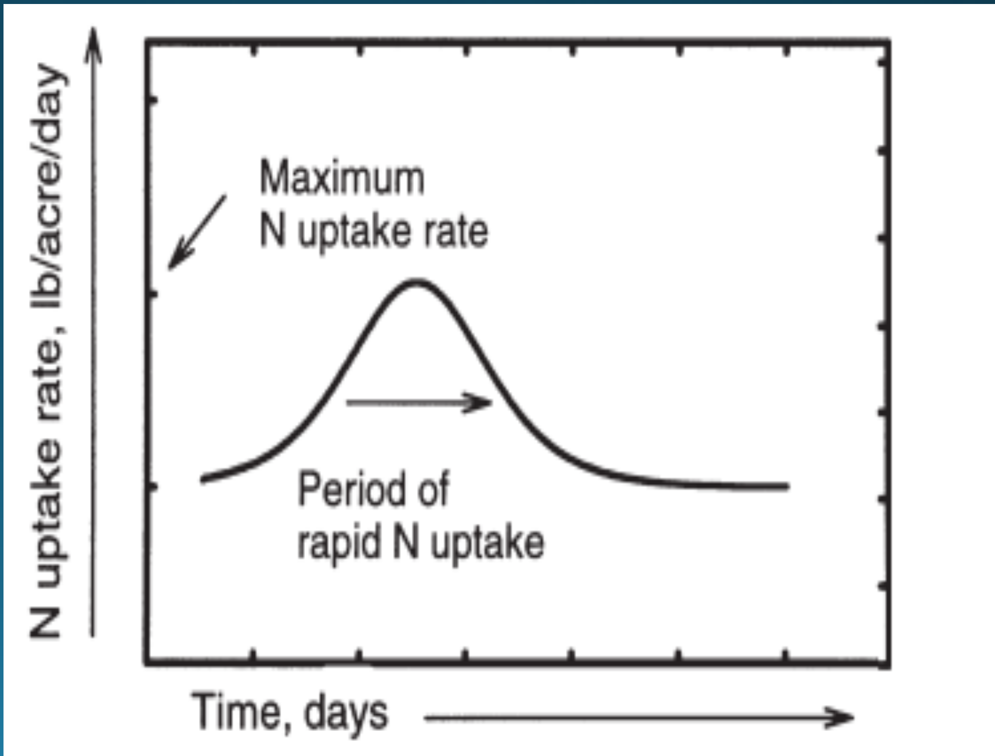

Gaseous ammonia released to the air
NH_3
NH_3
NH_2
NH_2
Urea molecule
C
O
Soil
If urea is hydrolyzed by urease at the **soil surface**, part of the NH_3 that is released may be lost to the air.


Soil
If urea is hydrolyzed by urease **within the soil**, the NH_3 that is released reacts with soil water to form NH_4^+, which adheres to soil components.
NH_4^+
NH_4^+
NH_3
NH_3
NH_2
NH_2
C
O
NH_4^+ binds to soil.
Urea molecule

Timing of N availability

- Seedling stage: N demand small
- Rapid vegetative growth (4 to 6 weeks): High N demand
- Reproductive growth (flowering): N moves from leaves to seed
  - Little or no additional N uptake

- What time is most efficient for N fertilization?



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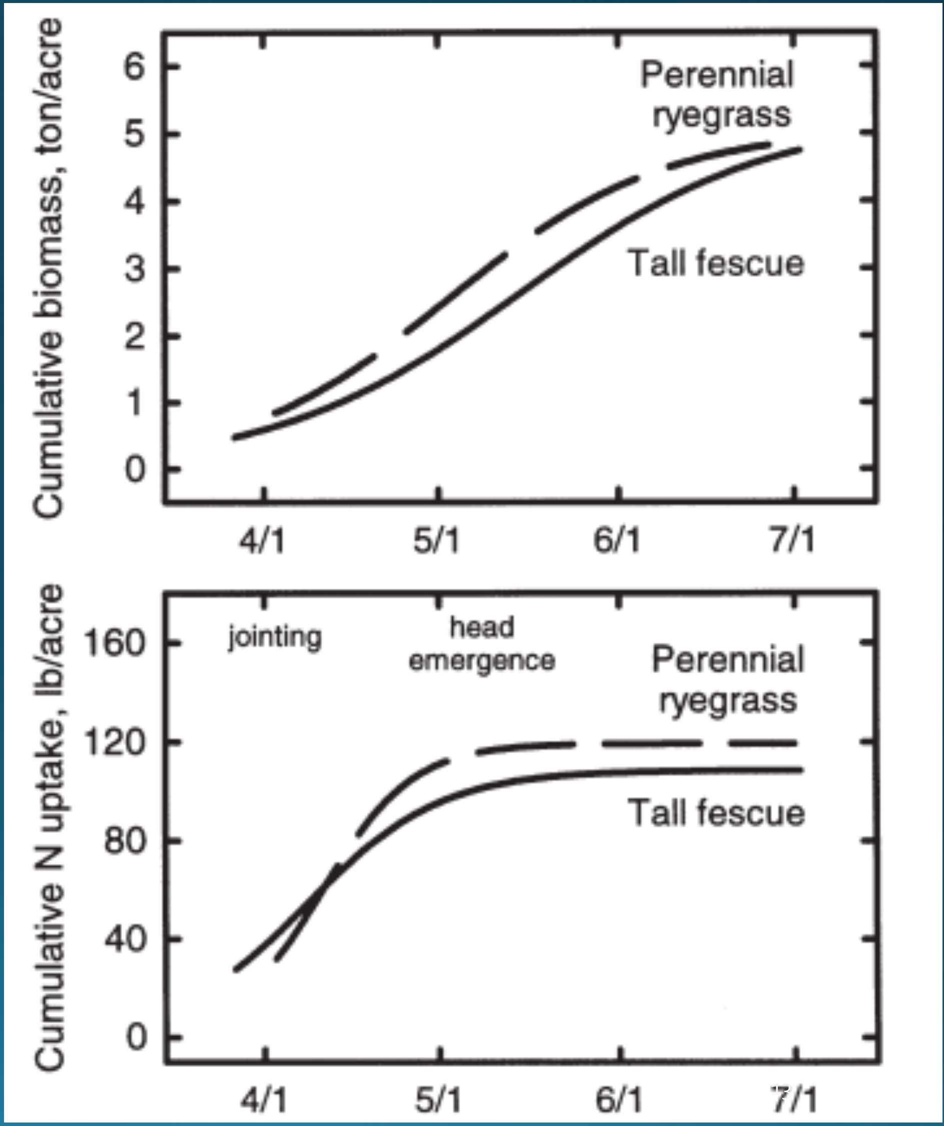


cereal rye cover crop scavenges nitrate over the winter

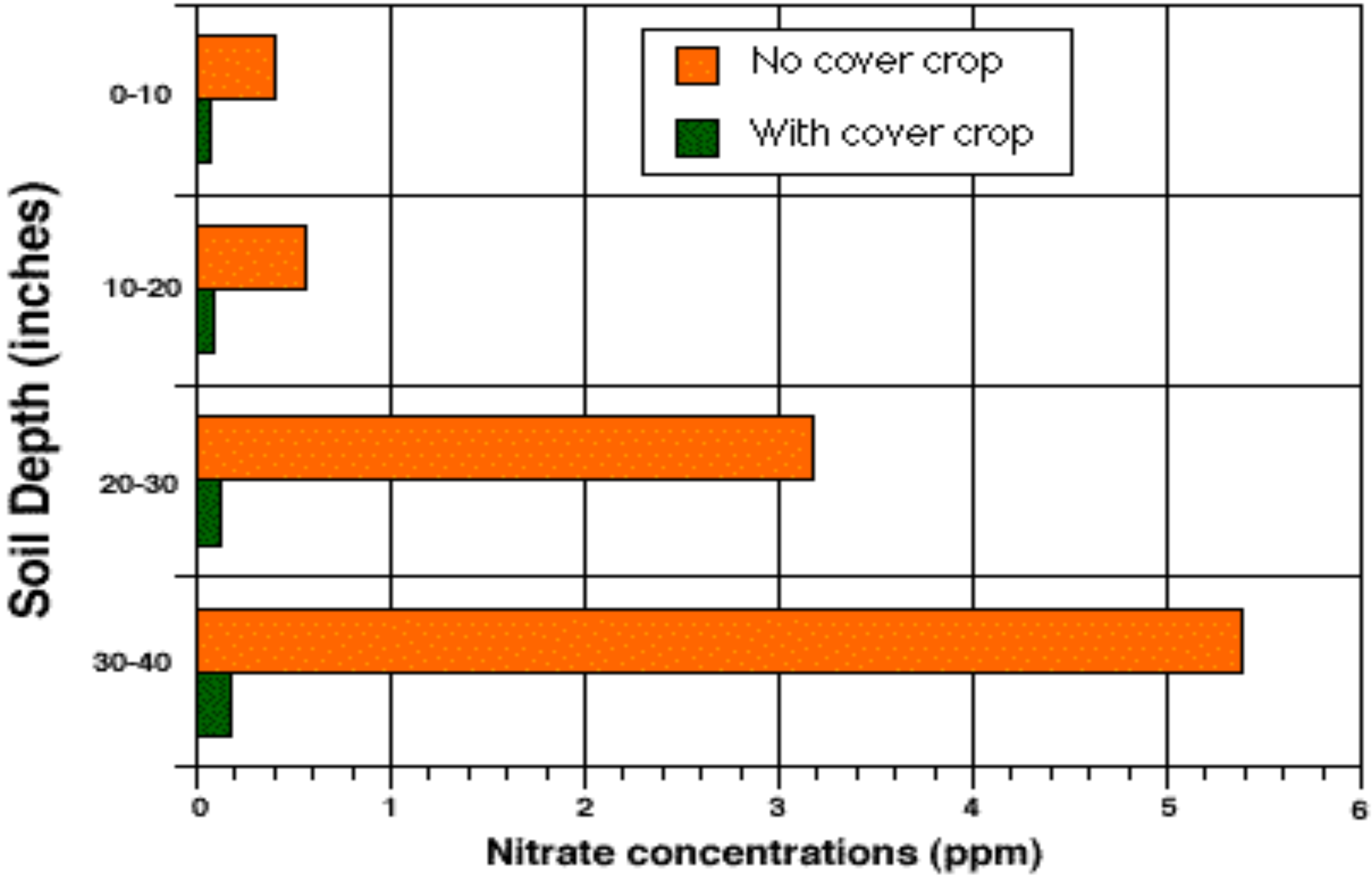


Figure 3. Effect of a cereal rye cover crop on soil nitrate concentrations (ppm) in broccoli plots fertilized the previous spring with 250 pounds N/acre. Samples were taken April 15, 1992. (Data from Hemphill and Hart, 1993.)

Part 2

Estimated Average Rates of Biological N_2 Fixation

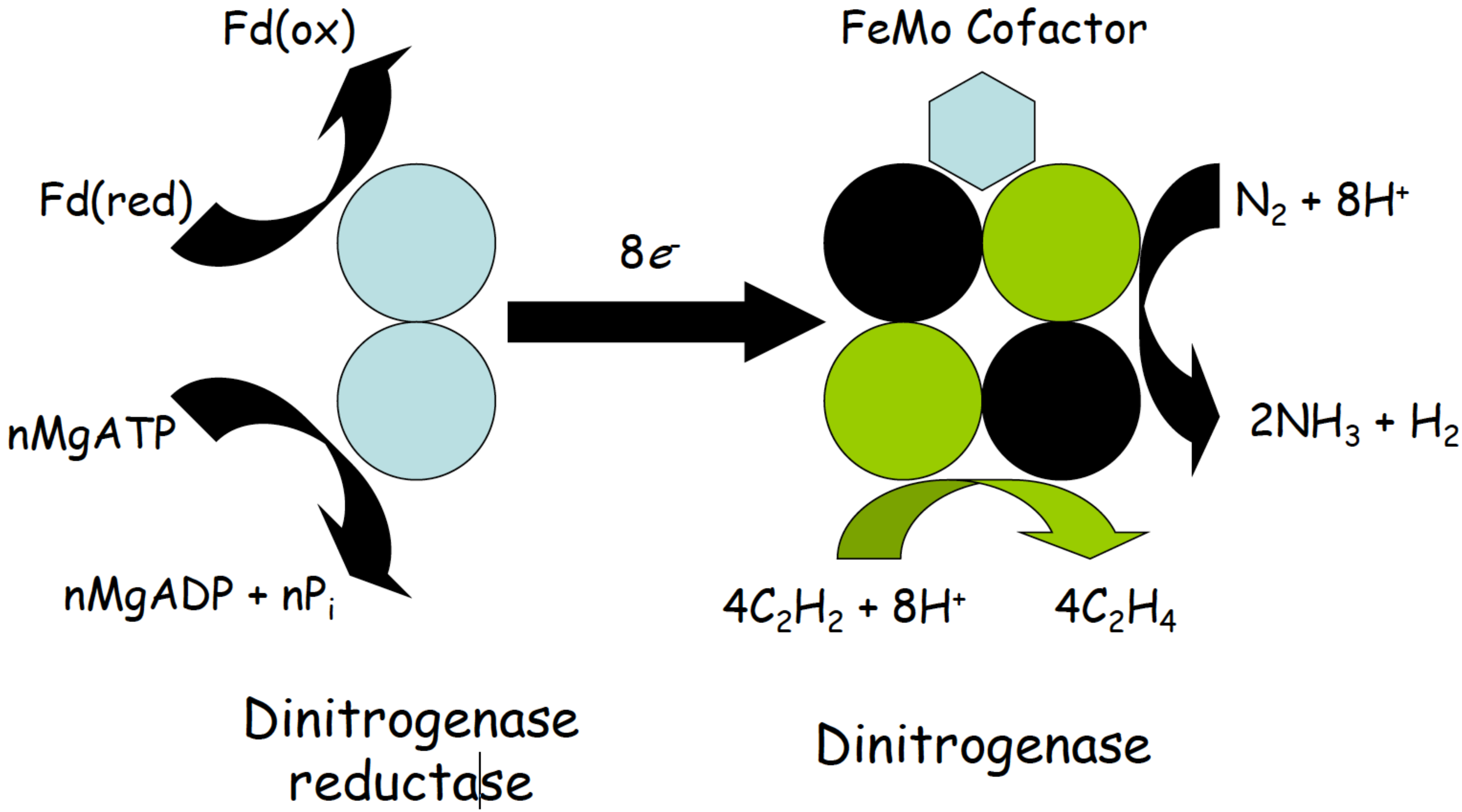
Organism or system	N_2 fixed ($kg\ ha^{-1}\ y^{-1}$)
Free-living microorganisms	
Cyanobacteria	25
Azotobacter	0.3
Clostridium pasteurianum	0.1-0.5
Grass-Bacteria associative symbioses	
Azospirillum	5-25
Cyanobacterial associations	
Gunnera	10-20
Azolla	50
Lichens	40-80
Leguminous plant symbioses with rhizobia	
Grain legumes (*Glycine, Vigna, Lespedeza, Phaseolus*)	20-180
Pasture legumes (*Trifolium, Medicago, Lupinus*)	50-300
Actinorhizal plant symbioses with *Frankia*	
Alnus	40-300
Hippophaë	1-150
Ceanothus	1-50
Coriaria	50-150
Casuarina	50

Nitrogen Fixation Process

Energetics

- $N \equiv N$
- Haber-Bosch (100-200 atm, 400-500°C, ~1,900 kJ kg^{-1} N)
- Nitrogenase (~950 kJ kg^{-1} N)

Nitrogenase



$$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16MgADP$$

Genetics of Nitrogenase

Gene	Properties and function
nifH	Dinitrogenase reductase
nifDK	Dinitrogenase
nifA	Regulatory, activator of most *nif* and *fix* genes
nifB	FeMo cofactor biosynthesis
nifEN	FeMo cofactor biosynthesis
nifS	Unknown
fixABCX	Electron transfer
fixK	Regulatory
fixLJ	Regulatory, two-component sensor/effector
fixNOQP	Electron transfer
fixGHIS	Transmembrane complex

Types of Biological Nitrogen Fixation

Free-living (asymbiotic)

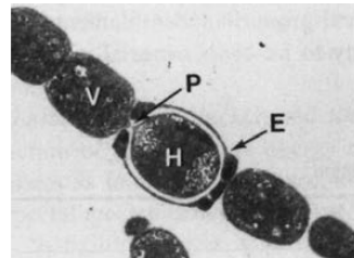
- Cyanobacteria
- *Azotobacter*

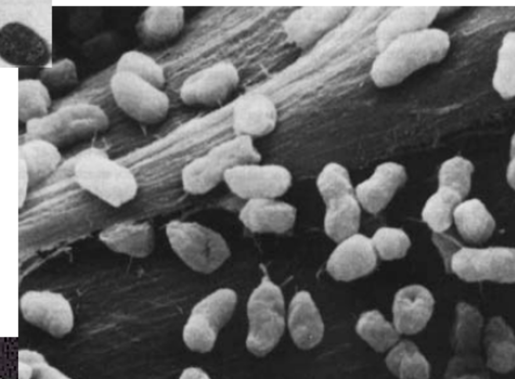
Associative

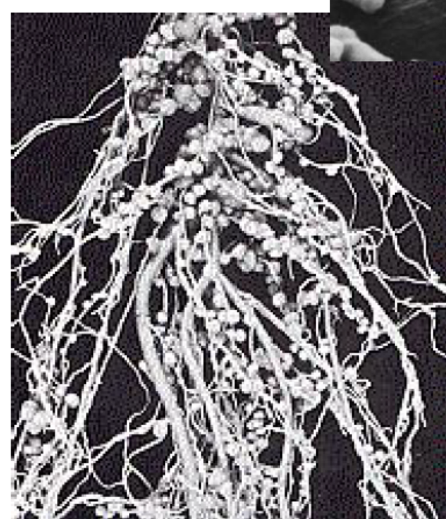
- Rhizosphere–*Azospirillum*
- Lichens–cyanobacteria
- Leaf nodules

Symbiotic

- Legume-rhizobia
- Actinorhizal-*Frankia*







Free-living N_2 Fixation

Energy

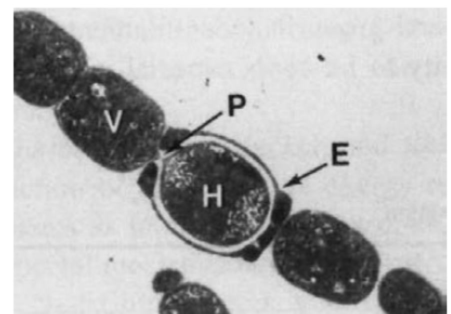
- 20-120 g C used to fix 1 g N

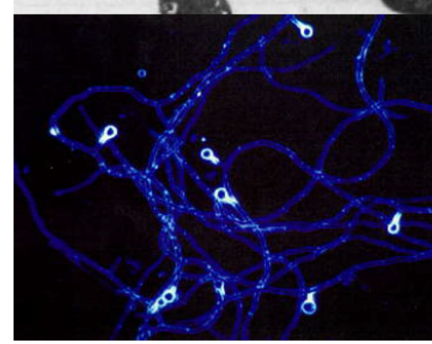
Combined Nitrogen

- *nif* genes tightly regulated
- Inhibited at low NH_4^+ and NO_3^- (1 µg g^{-1} soil, 300 µM)

Oxygen

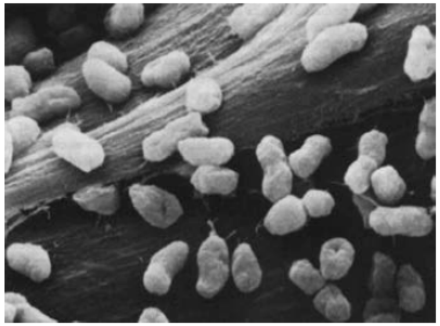
- Avoidance (anaerobes)
- Microaerophilly
- Respiratory protection
- Specialized cells (heterocysts, vesicles)
- Spatial/temporal separation
- Conformational protection





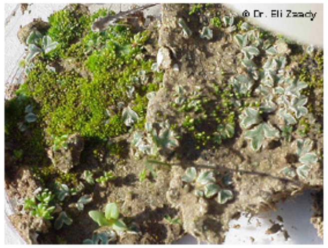
Associative N_2 Fixation

- Phyllosphere or rhizosphere (tropical grasses)
- *Azospirillum, Gluconacetobacter*
- 1 to 10% of rhizosphere population
- Some establish within root
- Same energy and oxygen limitations as free-living
- *Gluconacetobacter diazotrophicus* lives in internal tissue of sugar cane, grows in 30% sucrose, can reach populations of 10^6 to 10^7 cells g^{-1} tissue, and fix 100 to 150 kg N ha^{-1} y^{-1}



Phototrophic N_2-fixing Associations

- Lichens–cyanobacteria and fungi
- Mosses and liverworts — some have associated cyanobacteria



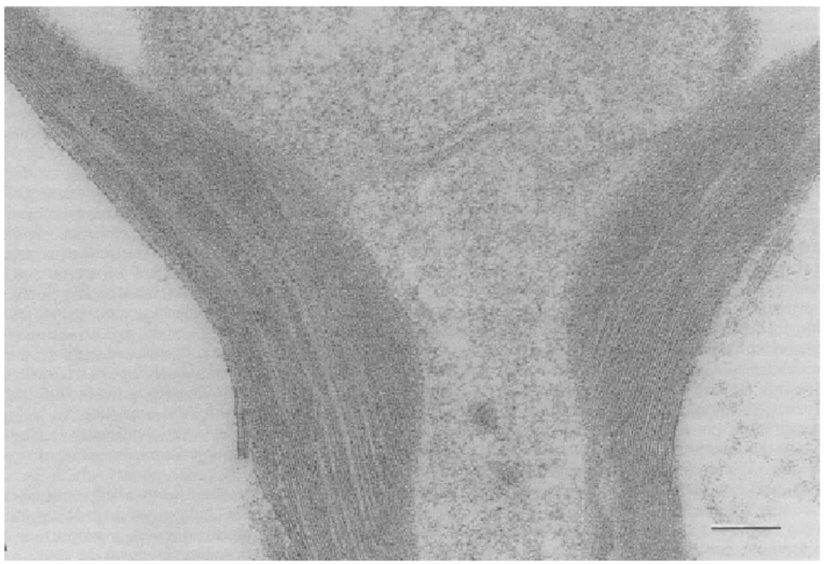
- *Azolla-Anabaena (Nostoc)* — cyanobacteria in stem of water fern

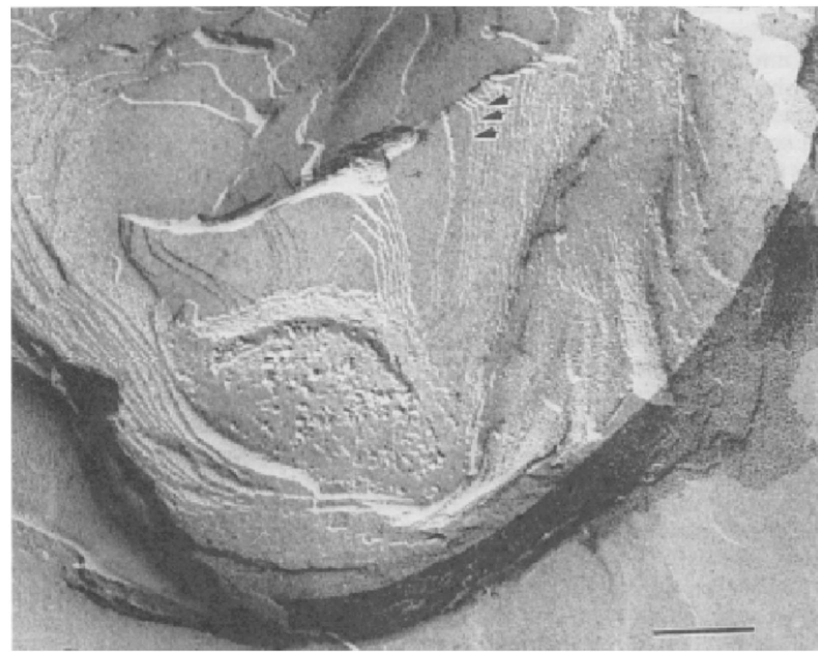
- *Gunnera-Nostoc* — cyanobacteria in stem nodule of dicot

- *Cycas-Nostoc* — cyanobacteria in roots of gymnosperm

Frankia and Actinorhizal Plants

- Actinobacteria (Gram +, filamentous); septate hyphae; spores in sporangia; thick-walled vesicles



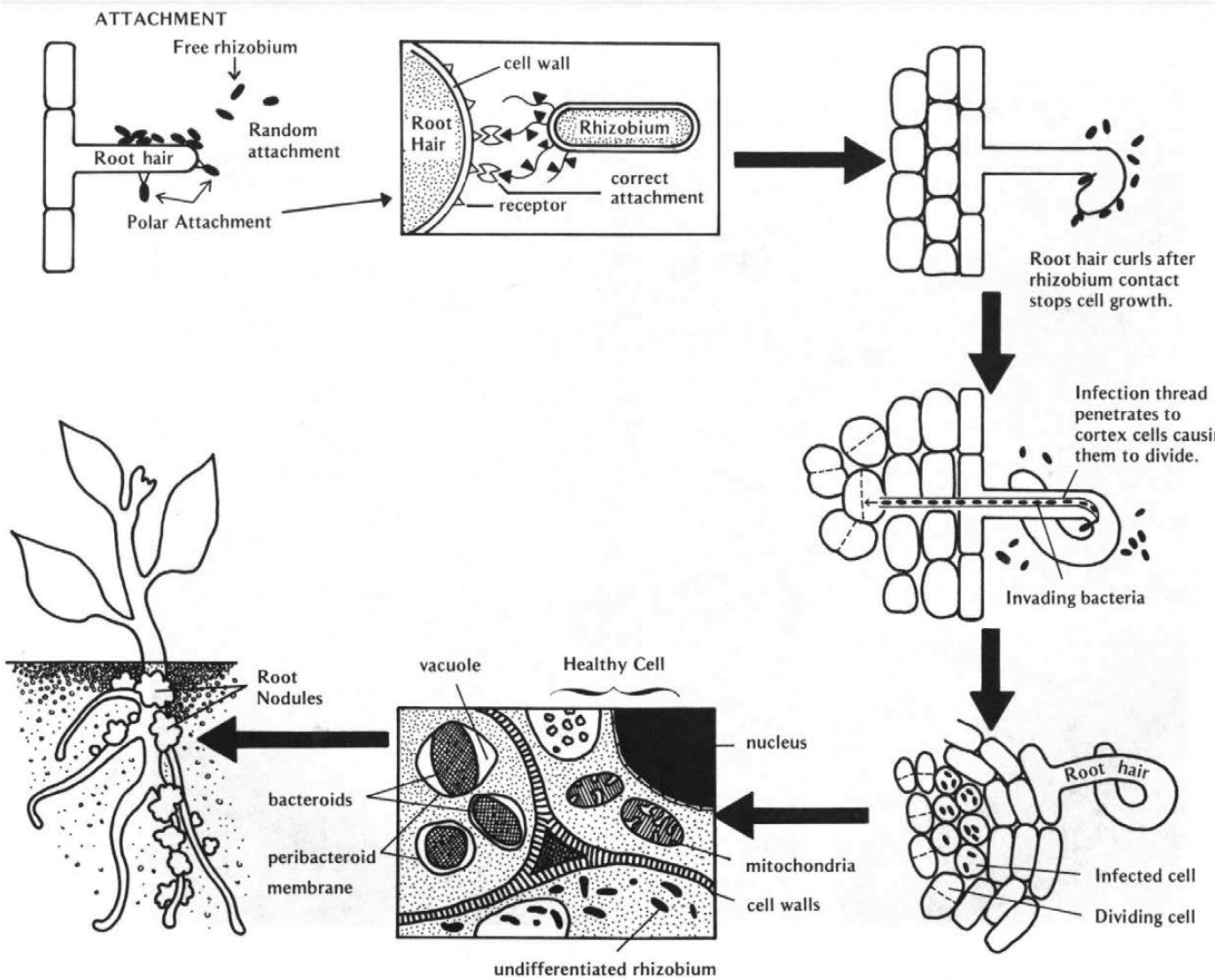


Frankia vesicles showing thick walls that confer protection from oxygen. Bars are 100 nm.

Actinorhizal Plant Hosts

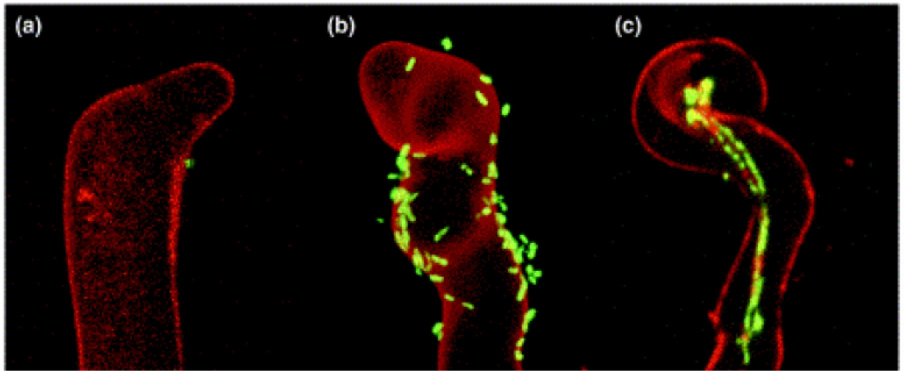
Family	Genera
Betulaceae	*Alnus*
Casuarinaceae	*Allocasuarina, Casuarina, Ceuthostoma, Gymnostoma*
Myricaceae	*Comptonia, Myrica*
Elaeagnaceae	*Elaeagnus, Hippophaë, Shepherdia*
Rhamnaceae	*Ceanothus, Colletia, Discaria, Kentrothamnus, Retanilla, Talguenea, Trevoa*
Rosaceae	*Cercocarpus, Chamaebatia, Cowania, Dryas, Purshia*
Coriariaceae	*Coriaria*
Datiscaceae	*Datisca*

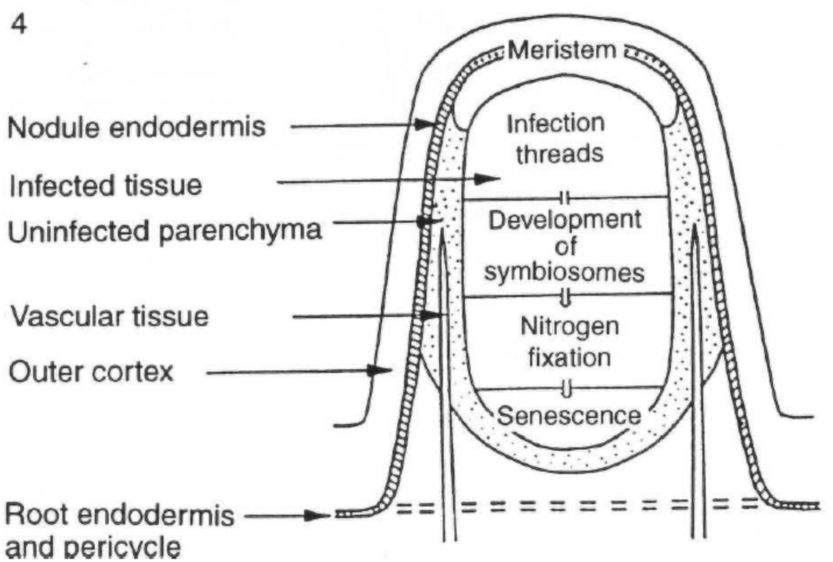
Nodulation in Legumes



Infection Process

- Attachment
- Root hair curling
- Localized cell wall degradation
- Infection thread
- Cortical cell differentiation
- Rhizobia released into cytoplasm (symbiosome formation)
- Bacteroid differentiation
- Induction of nodulins

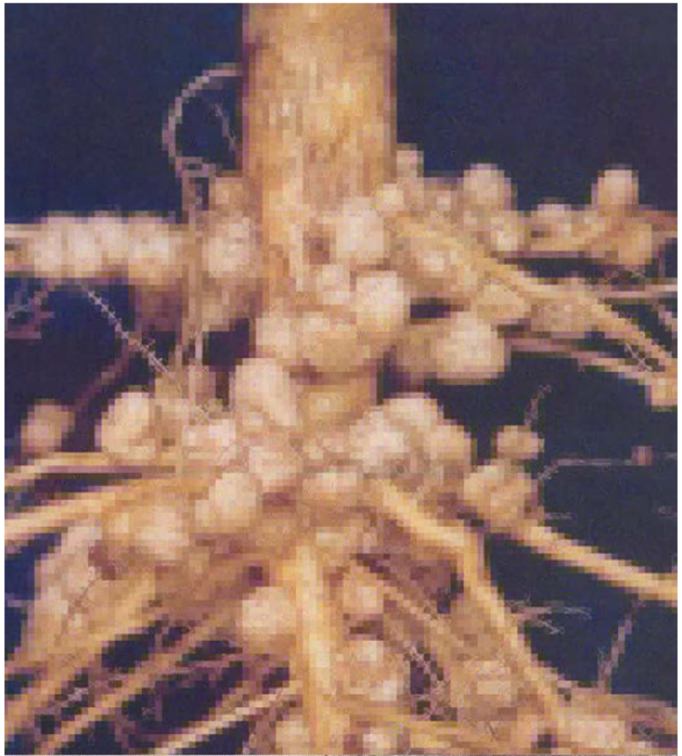


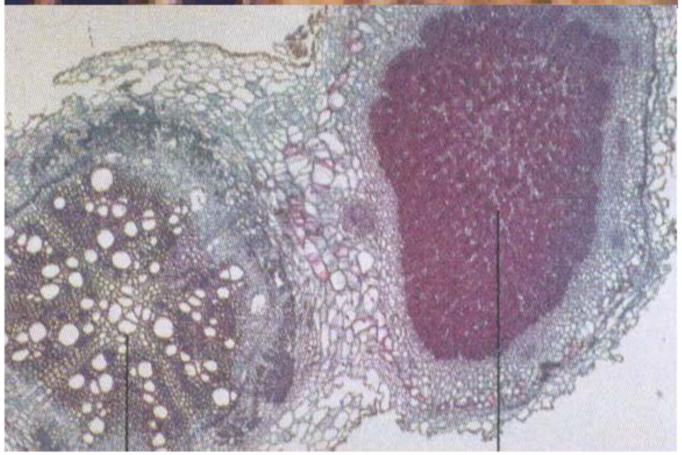


Indeterminate (pea) nodule

Legume-*Rhizobium* Symbiosis

- The subfamilies of legumes (Caesalpinioideae, Mimosoideae, Papilionoideae), 700 genera, and 19,700 species of legumes
- Only about 15% of the species have been evaluated for nodulation
- *Rhizobium*
  - Gram -, rod
  - Most studied symbiotic N_2-fixing bacteria
  - Now subdivided into several genera
  - Many genes known that are involved in nodulation (*nod, nol, noe* genes)





Taxonomy of Rhizobia

Genus	Species	Host plant
Rhizobium (~30 species)	*leguminosarum bv. trifolii*	*Trifolium* (clovers)
	" *bv. viciae*	*Pisum* (peas), *Vicia* (field beans), *Lens* (lentils)
	" *bv. phaseoli*	
	tropici	*Phaseolus* (bean), *Leucaena*
	etli	*Phaseolus* (bean)
	galegae	*Galegae* (goat's rue)
Ensifer (Sinorhizobium, ~10 species)	*meliloti*	*Medicago* (alfalfa)
	fredii	*Glycine* (soybean)
	saheli	*Sesbania*
	americanum	*Acacia*
Bradyrhizobium (~13 species)	*japonicum*	*Glycine* (soybean)
	elkanii	*Glycine* (soybean)
	liaoningense	*Glycine* (soybean)
Azorhizobium (2 species)	*caulinodans*	*Sesbania* (stem nodule)
Mesorhizobium (~20 species)	*loti*	*Lotus* (trefoil)
	huakuii	*Astragalus* (milkvetch)
	ciceri	*Cicer* (chickpea)
	mediterraneum	*Cicer* (chickpea)
Allorhizobium	*undicola*	*Neptunia*
Photorhizobium	spp.	*Aeschynomene* (stem nodule)